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(54) Title: ISOALLOXAZINE DERIVATIVES TO NEUTRALIZE BIOLOGICAL CONTAMINANTS

(57) Abstract: Methods are provided for neutralization of microorganisms in fluids or on surfaces. Preferably the fluids contain blood or blood products and comprise biologically active proteins. Preferred methods include the steps of adding an activation-effective amount of a microorganism neutralizer with an isoalloxazine backbone to a fluid and exposing the fluid to a triggering event. Preferred triggering events include light of a suitable wavelength and intensity to activate the microorganism neutralizer or a pH sufficient to activate the microorganism neutralizer. Other fluids, including juices, water and the like, may also be decontaminated by these methods as may surfaces of foods, animal carcasses, wounds, food preparation surfaces and bathing and washing vessel surfaces. Compounds with an isoalloxazine backbone are also provided.

ISOALLOXAZINE DERIVATIVES TO NEUTRALIZE BIOLOGICAL CONTAMINANTS

BACKGROUND OF THE INVENTION

Contamination of blood supplies with infectious microorganisms such as HIV, hepatitis and other viruses and bacteria presents a serious health hazard for those who must receive transfusions of whole blood or administration of various blood components such as platelets, red cells, blood plasma, Factor VIII, plasminogen, fibronectin, anti-thrombin III, cryoprecipitate, human plasma protein fraction, albumin, immune serum globulin, prothrombin complex plasma growth hormones, and other components isolated from blood. Blood screening procedures currently available may miss contaminants. Thus, there is a need for sterilization procedures that effectively neutralize all infectious viruses and other microorganisms but do not damage cellular blood components, do not degrade desired biological activities of proteins, and preferably do not need to be removed prior to administration of the blood product to the patient.

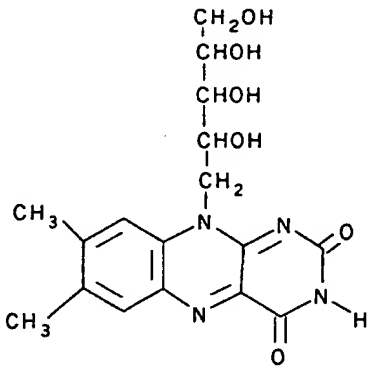
The use of photosensitizers, compounds which absorb light of a defined wavelength and transfer the absorbed energy to an energy acceptor, has been proposed for blood component sterilization. Various photosensitizers have been proposed for use as blood additives. A review of some photosensitizers including psoralens, and some of the issues of importance in choosing photosensitizers for decontamination of blood products is provided in Goodrich, R.P., et al. (1997), "The Design and Development of Selective, Photoactivated Drugs for Sterilization of Blood Products," Drugs of the Future 22:159-171.

Some photosensitizers that have been proposed for use for blood component sterilization have undesirable properties. For example, European Patent Application 196,515 published October 8, 1986, suggests the use of non-endogenous photosensitizers such as porphyrins, psoralens, acridine, toluidines, flavine (acriflavine hydrochloride), phenothiazine derivatives, and dyes such as neutral red and methylene blue, as blood additives. Another

molecule, chlorpromazine, has been used as a photosensitizer; however its usefulness is limited by the fact that it should be removed from any fluid administered to a patient after the decontamination procedure because it has a sedative effect. Protoporphyrin, which occurs naturally within the body, can be metabolized to form a photosensitizer; however, its usefulness is limited in that it degrades the desired biological activities of proteins.

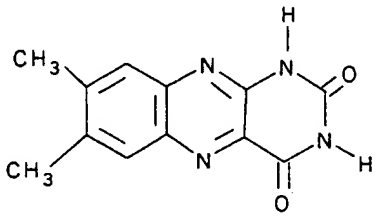
In addition to molecules which can serve as photosensitizers, alkylating agents have been proposed for use as blood contaminant neutralizers. Alkylating agents are believed to deactivate microorganisms by alkylating nucleophilic groups of amino acid residues and nucleic bases at a certain pH. Ethyleneimine has been reported to deactivate certain viruses (United States Patent No. 5,891,075 (Budowsky, et al.),WO 97/07674 (published March 6, 1997)).

United States Patent Application Number 09/119,666 and continuation in part 09/357,188, hereby incorporated by reference to the extent not inconsistent with the disclosure herein, describes methods and apparatus for neutralization of biological contaminants using endogenous photosensitizers, including 7,8-dimethyl-10-ribityl isoalloxazine (riboflavine).



7,8-dimethyl-10-ribityl isoalloxazine

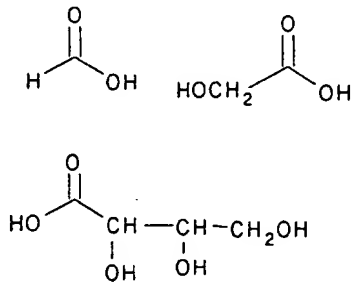
7,8-dimethyl-10-ribityl isoalloxazine (Riboflavine or vitamin B2) absorbs light from about 200 to 500 nm. The ring system core of 7,8-dimethyl-10-ribityl isoalloxazine is resistant to photodegradation but the ribityl side chain of riboflavin undergoes photodegradation. Photolysis of 7,8-dimethyl-10-ribityl isoalloxazine may form lumichrome (7,8-dimethylalloxazine) depending on conditions. 7,8-dimethylalloxazine strongly absorbs ultraviolet (UV) light and only weakly absorbs visible light.



7,8-dimethylalloxazine

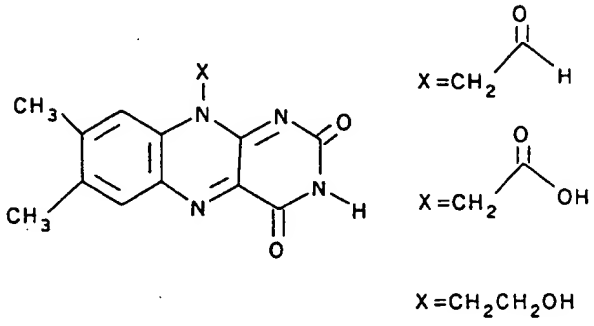
United States Patent No. 5,811,144 discusses the treatment of beer with visible light under substantially anaerobic conditions to reportedly reduce the riboflavin content of the beer.

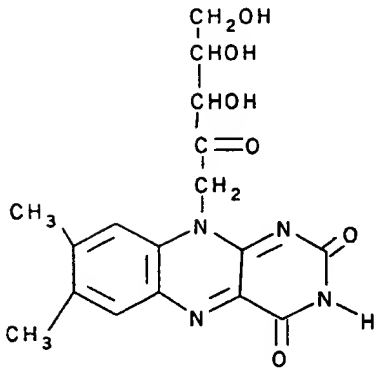
Small molecules such as those shown below which are derived from the ribityl side chain are expected to be products from the photolysis of riboflavin.

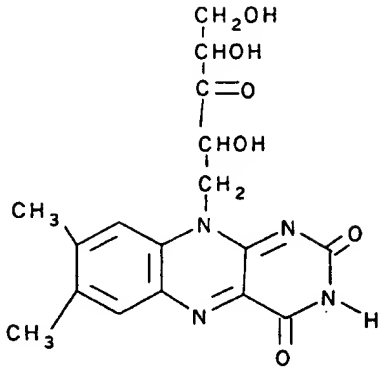


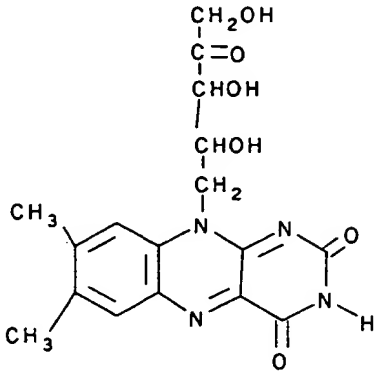
Incomplete photolysis of riboflavin leads to isoalloxazine-containing intermediates (Smith, E.C. and Metzler, D.E. (1963) J. Am. Chem. Soc. **85**:3285-3288; Carins, W.L. and Metzler,

D.E. (1971) J. Am. Chem. Soc. 93:2772-2777; Treadwell, G.E. et al. (1968) J. Chromatog. 35:376-388). Some of the identified compounds are:

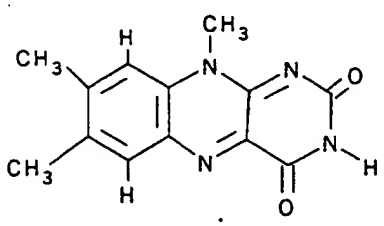








These compounds absorb visible light and may convert to either lumichrome or another riboflavin metabolite, lumiflavin (7,8,10-trimethylisoalloxazine) upon complete photolysis, depending on the experimental conditions.



7,8,10-trimethylisoalloxazine

Lumichrome and lumiflavin are reported to be produced by the photolysis of milk (Parks, O.W. and Allen, C. (1977) Dairy Sci. 60:1038-1041; Toyosaki, T. and Hayashi, A. (1993) Milewissenschaft 48:607-609).

As a result of the degradation of 7,8-dimethyl-10-ribityl isoalloxazine upon exposure to light, a combination of visible and ultraviolet light is preferred in decontamination procedures using 7,8-dimethyl-10-ribityl isoalloxazine. Since UV light has a higher energy per photon than visible light, and because UV light is absorbed more strongly than visible light by useful compounds in the biological fluid, more damage to the useful components in the biological fluid containing the contaminants will occur when ultraviolet light is used in combination with visible light than when visible light can be used alone.

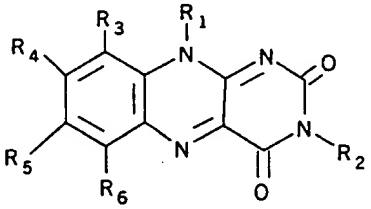
There is a need for compounds that neutralize microorganisms with visible light alone.

All publications referred to herein are hereby incorporated by reference to the extent not inconsistent with the disclosure herein.

BRIEF SUMMARY OF THE INVENTION

Methods are provided for treating a fluid or other material to neutralize at least some of the microorganisms and white cells which may be present therein or thereon. Such fluids may also contain one or more components selected from the group consisting of protein, e.g. biologically active protein such as a therapeutic protein, blood and blood constituents, without destroying the biological activity of such components. The methods comprise:

(a) mixing a neutralization-effective amount of a microorganism neutralizer of formula:



with the fluid, wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing, and $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20;

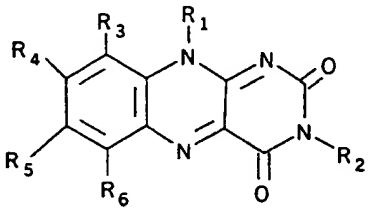
provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O and R1, R4 and R5 are not all methyl groups when R2, R3 and R6 are all hydrogen;

(b) exposing the fluid to a triggering event, whereby at least some of the microorganisms are neutralized.

In one group of compounds, n is an integer between 0 and 5. In another group of compounds, n is an integer from 0 to 10. In another group of compounds, n is an integer from 0 to 20.

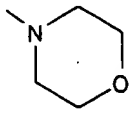
A fluid is provided comprising biologically active protein, blood or blood constituents, and microorganism neutralizer, made by the method above. The fluid may also contain neutralized microorganisms. A blood product is also provided comprising a microorganism neutralizer made by the method above.

Compounds are provided having the structure:



wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing, and $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b andR^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20; provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O; and R1 is not a 2-, 3-, 4- or 5-carbon straight chain alkyl that terminates in -OH, -COH, or -H when R2, R3 and R6 are H, and R4 and R5 are CH_3; R1 is not - $CH_2CH_2-(CHOH)_2-CH_3$ or $-CH_2CH_2-(CHOH)_2-CH_2SO_4$ or 1'-D-sorbityl or 1'-D-dulcityl or 1'-D-rhamnityl or 1'-D,L-glyceryl or $-CH_2-O-C(O)-CH_3$ or

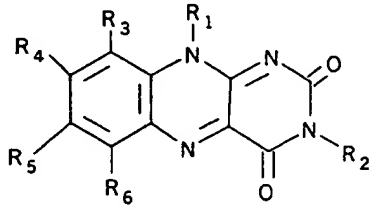
$-CH_2-O-C(O)-CH_2CH_3$ or 2', 3', 4', 5'-di-O-isopropyridene-riboflavin or 8-aminooctyl when R2, R3 and R6 are H and R4 and R5 are CH_3; R1 is not 1'-D-sorbityl or 1'-D-dulcityl when R4 and R5 are both chlorines and when R2, R3 and R6 are all hydrogens; R5 is not ethyl or chloro when R1 and R4 are methyl and R2, R3 and R6 are all hydrogens; R4 and R5 are not both methoxy or both tetramethylene when R1 is methyl and R2, R3 and R6 are all hydrogens; R2 is not $-CH_2CH_2NH$ when R1, R4 and R5 are CH_3 and R3 and R6 are H; R2 is not



when R1, R4 and R5 are CH_3 and R3 and R6 are H; R5 is not chloro when R4 is methoxy and R1 is ethyl-2'N-pyrrolidino and R2, R3, and R6 are hydrogen; R1 is not N,N-dimethylaminopropyl or N,N-diethylaminoethyl when R5 is chloro or methyl and R2, R3, R4 and R6 are hydrogen; R3 is not $-NH(CH_2CH_2)Cl$ when R6 is $-NH_2$ and R1, R2, R4 and R5 are H; R1, R4, R5 are not all methyl groups when all of R2, R3 and R6 are hydrogens; R1, R4, R5 and R2 are not all methyl groups when R3 and R6 are hydrogens; R2 is not carboxymethyl when R1, R4 and R5 are methyl and R3 and R6 are hydrogen; R4 is not $-NH_2$ when R1 and R5 are methyl and R2, R3 and R6 are all hydrogen; R1 is not a phenyl group when R4 and R5 are methyl and R2, R3 and R6 are all H; R1 is not methyl or N,N-dimethylaminoethyl when all of R2, R3, R4, R5 and R6 are hydrogen; R2, R4, R5 are not all methyl when R1 is acetoxyethyl and R3 and R6 are hydrogen; R5 is not methyl when R1 is N,N-diethylaminoethyl and R2, R3, R4 and R6 are all hydrogen; R4 and R5 are not both chlorine when R1 is methyl and R2, R3 and R6 are all hydrogen; R1 is not ethyl, β-chloroethyl, n-butyl, anilino, benzyl, phenyl, p-tolyl or p-anisyl when R5 is NH_2 and R2, R3, R4 and R6 are all hydrogen; and R4 is not chlorine when R1 is N,N-dimethylaminopropyl and R2, R3, R5 and R6 are all hydrogen.

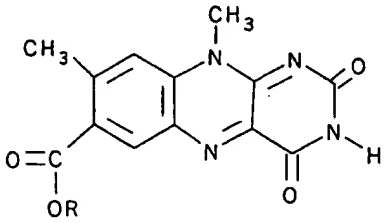
In one group of compounds, n is an integer between 0 and 5. In another group of compounds, n is an integer from 0 to 10. In another group of compounds, n is an integer from 0 to 20.

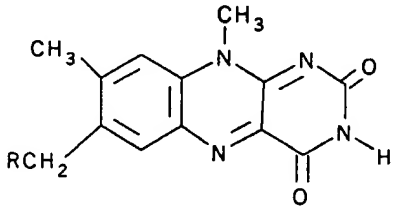
Compounds containing any combination of substituents or members of the Markush groups specified above are within the scope of the invention. All compounds of the invention have the ability to neutralize microorganisms. All substituents of the compounds of the invention may be the same, all substituents may be different, or any combination of substituents may be the same or different. Substituents with a specified function, for example those that impart water solubility to the compound, may be included at any of R1-R6. Compounds of the invention include all those compounds with the isoalloxazine backbone (shown below):

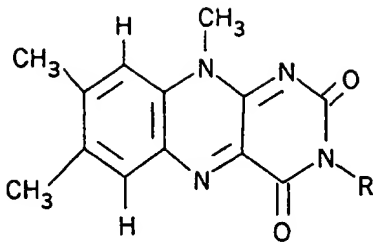


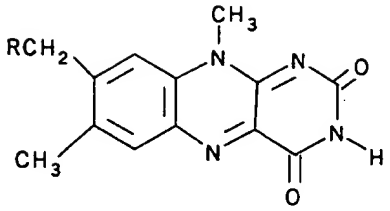
where R1-R6 are substituted with various substituents, as described elsewhere, except those previously known to the art. The substituents included in the compounds and used in the methods of the invention may be any substituent not having structures or reactivity which would substantially interfere with the desired microorganism neutralization of the microorganism neutralizer, as may readily be determined without undue experimentation by those skilled in the art.

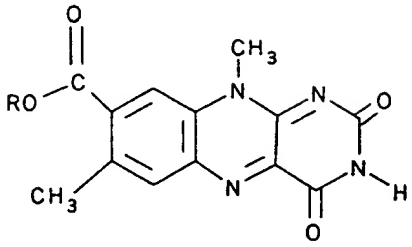
The invention provides a class of compounds wherein a plurality of R1, R2, R3, R4, R5 and R6 are neither CH_3 nor H; and a class of compounds wherein one of R1, R2, R3, R4, R5 and R6 is neither CH_3 nor H. Particular embodiments of compounds of those classes include those wherein a R1, R2, R3, R4, R5 or R6 which is neither CH_3 nor H imparts substantial water solubility to the microorganism neutralizer. Preferred examples of these compounds are:







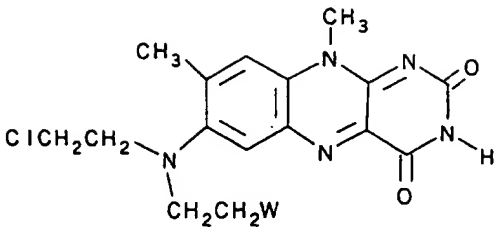


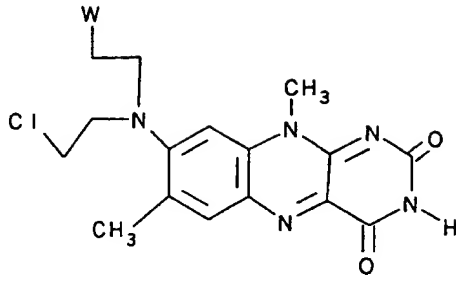


wherein R is a substituent imparting water solubility to the molecule, including, but not limited to, ascorbate, alcohol, polyalcohol; amine or polyamines, straight chain or cyclic saccharides, sulfates, phosphates, alkyl chains optionally substituted with -OH at any position, glycols, including polyethylene glycol and polyethers.

Another class of compounds of the invention include those wherein a R1, R2, R3, R4, R5 or R6 that is neither H nor CH_3 contains a halogen or is a halogen, wherein the halogen is selected from the group consisting of fluorine, chlorine, bromine and iodine. Particular embodiments of compounds of this class include compounds where a R1, R2, R3, R4, R5 or R6 that is neither H nor CH_3 is: $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, or is a water soluble group, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen and optionally substituted hydrocarbyl, and n is an integer from 0 to 20.

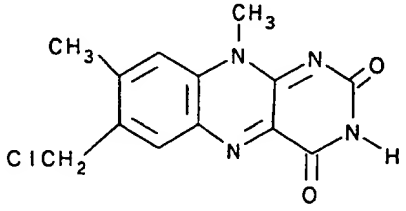
Preferred examples of compounds of this class are:





where W is a substituent imparting water solubility to the molecule, including, but not limited to, ascorbate, alcohol, polyalcohol; amine or polyamines, straight chain or cyclic saccharides, sulfates, phosphates, alkyl chains optionally substituted with -OH at any position, glycols, including polyethylene glycol and polyethers.

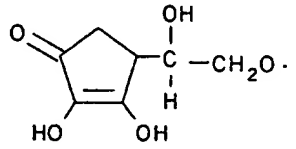
Another particular embodiment of compounds wherein a R1, R2, R3, R4, R5 or R6 that is neither H nor CH_3 contains a halogen or is a halogen includes compounds wherein a R1, R2, R3, R4, R5 or R6 that is neither H nor CH_3 is: $X-(CH_2)_n-$, wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 6. A preferred example of compounds of this class include:



Other classes of compounds of this invention include those wherein R1 is CH_2-$(CH_2OH)_3$-CH_2OH and those wherein R1 is not CH_2-$(CH_2OH)_3$-CH_2OH. Also, those compounds wherein R3 and R6 are H are included in the invention.

DEFINITIONS

A "carbonyl compound" is any compound containing a carbonyl group (-C=O). The term "amine" refers to a primary, secondary, or tertiary amine group. A "polyamine" is a group that contains more than one amine group. A "sulfate" group is a salt of sulfuric acid. Sulfate groups include the group $(SO_4)^{2-}$. "Phosphates" contain the group PO_4^{3-}. "Glycols" are groups that have two alcohol groups per molecule of the compound. "Glycols" are also known as diols. A glycol is described by the formula: $C_nH_{2n}(OH)_2$, where n is an integer. An "aldehyde" is a group containing the formula -(C=O)-H. A "ketone" is a group with formula R-(C=O)-R, where R is not hydrogen. The R groups on ketones do not need to be the same. A "carboxylic acid" is a group which includes the formula: -COOH. An "ether" is a group containing -O-. A "salt" is a group where a hydrogen atom of an acid has been replaced with a metal atom or a positive radical, such as NH_4^+. "Ascorbate" includes groups with formula:



The term "hydrocarbyl" is used herein to refer generally to organic groups comprised of carbon chains to which hydrogen and optionally other elements are attached. CH_2 or CH groups and C atoms of the carbon chains of the hydrocarbyl may be replaced with one or more heteroatoms (i.e., non-carbon atoms). Suitable heteroatoms include but are not limited to O, S, P and N atoms. The term hydrocarbyl includes, but is not limited to alkyl, alkenyl, alkynyl, ether, polyether, thioether, straight chain or cyclic saccharides, ascorbate, aminoalkyl, hydroxylalkyl, thioalkyl, aryl and heterocyclic aryl groups, optionally substituted isoalloxazine molecules, amino acid, polyalcohol, glycol, groups which have a mixture of saturated and unsaturated bonds, carbocyclic rings and combinations of such groups. The term also includes straight-chain, branched-chain and cyclic structures or combinations thereof. Hydrocarbyl groups are optionally substituted. Hydrocarbyl substitution includes

substitution at one or more carbons in the group by moieties containing heteroatoms. Suitable substituents for hydrocarbyl groups include but are not limited to halogens, including chlorine, fluorine, bromine and iodine, OH, SH, NH_2, COH, CO_2H, OR_a, SR_a, NR_aR_b, $CONR_aR_b$, where R_a and R_b independently are alkyl, unsaturated alkyl or aryl groups.

The term "alkyl" takes its usual meaning in the art and is intended to include straight-chain, branched and cycloalkyl groups. The term includes, but is not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, neopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,1-dimethylpropyl, n-hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 2-ethylbutyl, 1-ethylbutyl, 1,3-dimethylbutyl, n-heptyl, 5-methylhexyl, 4-methylhexyl, 3-methylhexyl, 2-methylhexyl, 1-methylhexyl, 3-ethylpentyl, 2-ethylpentyl, 1-ethylpentyl, 4,4-dimethylpentyl, 3,3-dimethylpentyl, 2,2-dimethylpentyl, 1,1-dimethylpentyl, n-octyl, 6-methylheptyl, 5-methylheptyl, 4-methylheptyl, 3-methylheptyl, 2-methylheptyl, 1-methylheptyl, 1-ethylhexyl, 1-propylpentyl, 3-ethylhexyl, 5,5-dimethylhexyl, 4,4-dimethylhexyl, 2,2-diethylbutyl, 3,3-diethylbutyl, and 1-methyl-1-propylbutyl. Alkyl groups are optionally substituted. Lower alkyl groups are C_1-C_6 alkyl and include among others methyl, ethyl, n-propyl, and isopropyl groups.

The term "cycloalkyl" refers to alkyl groups having a hydrocarbon ring, particularly to those having rings of 3 to 7 carbon atoms. Cycloalkyl groups include those with alkyl group substitution on the ring. Cycloalkyl groups can include straight-chain and branched-chain portions. Cycloalkyl groups include but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and cyclononyl. Cycloalkyl groups can optionally be substituted.

Aryl groups may be substituted with one, two or more simple substituents including, but not limited to, lower alkyl, e.g., methyl, ethyl, butyl; halo, e.g., chloro, bromo; nitro; sulfato; sulfonyloxy; carboxy; carbo-lower-alkoxy, e.g., carbomethoxy, carbethoxy; amino; mono- and di-lower-alkylamino, e.g., methylamino, ethylamino, dimethylamino, methylethylamino; amido; hydroxy; lower-alkoxy, e.g., methoxy, ethoxy; and lower-alkanoyloxy, e.g., acetoxy.

The term "unsaturated alkyl" group is used herein generally to include alkyl groups in which one or more carbon-carbon single bonds have been converted to carbon-carbon double or triple bonds. The term includes alkenyl and alkynyl groups in their most general sense. The term is intended to include groups having more than one double or triple bond, or combinations of double and triple bonds. Unsaturated alkyl groups include, without limitation, unsaturated straight-chain, branched or cycloalkyl groups. Unsaturated alkyl groups include without limitation: vinyl, allyl, propenyl, isopropenyl, butenyl, pentenyl, hexenyl, hexadienyl, heptenyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, ethynyl, propargyl, 3-methyl-1-pentynyl, and 2-heptynyl. Unsaturated alkyl groups can optionally be substituted.

Substitution of alkyl, cycloalkyl and unsaturated alkyl groups includes substitution at one or more carbons in the group by moieties containing heteroatoms. Suitable substituents for these groups include but are not limited to OH, SH, NH_2, COH, CO_2H, OR_c, SR_c, P, PO, NR_cR_d, $CONR_cR_d$, and halogens, particularly chlorines and bromines where R_c and R_d, independently, are alkyl, unsaturated alkyl or aryl groups. Preferred alkyl and unsaturated alkyl groups are the lower alkyl, alkenyl or alkynyl groups having from 1 to about 3 carbon atoms.

The term "aryl" is used herein generally to refer to aromatic groups which have at least one ring having a conjugated pi electron system and includes without limitation carbocyclic aryl, aralkyl, heterocyclic aryl, biaryl groups and heterocyclic biaryl, all of which can be optionally substituted. Preferred aryl groups have one or two aromatic rings.

"Carbocyclic aryl" refers to aryl groups in which the aromatic ring atoms are all carbons and includes without limitation phenyl, biphenyl and napthalene groups.

"Aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include among others benzyl, phenethyl and picolyl, and may be optionally substituted. Aralkyl groups include those with heterocyclic and carbocyclic aromatic moieties.

"Heterocyclic aryl groups" refers to groups having at least one heterocyclic aromatic ring with from 1 to 3 heteroatoms in the ring, the remainder being carbon atoms. Suitable heteroatoms include without limitation oxygen, sulfur, and nitrogen. Heterocyclic aryl groups include among others furanyl, thienyl, pyridyl, pyrrolyl, N-alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl, benzofuranyl, quinolinyl, and indolyl, all optionally substituted.

"Heterocyclic biaryl" refers to heterocyclic aryls in which a phenyl group is substituted by a heterocyclic aryl group ortho, meta or para to the point of attachment of the phenyl ring to the decalin or cyclohexane. Heterocyclic biaryl includes among others groups which have a phenyl group substituted with a heterocyclic aromatic ring. The aromatic rings in the heterocyclic biaryl group can be optionally substituted.

"Biaryl" refers to carbocyclic aryl groups in which a phenyl group is substituted by a carbocyclic aryl group ortho, meta or para to the point of attachment of the phenyl ring to the decalin or cyclohexane. Biaryl groups include among others a first phenyl group substituted with a second phenyl ring ortho, meta or para to the point of attachment of the first phenyl ring to the decalin or cyclohexane structure. Para substitution is preferred. The aromatic rings in the biaryl group can be optionally substituted.

Aryl group substitution includes substitutions by non-aryl groups (excluding H) at one or more carbons or where possible at one or more heteroatoms in aromatic rings in the aryl group. Unsubstituted aryl, in contrast, refers to aryl groups in which the aromatic ring carbons are all substituted with H, e.g. unsubstituted phenyl ($-C_6H_5$), or naphthyl ($-C_{10}H_7$). Suitable substituents for aryl groups include among others, alkyl groups, unsaturated alkyl groups, halogens, OH, SH, NH_2, COH, CO_2H, OR_e, SR_e, NR_eR_f, $CONR_eR_f$, where R_e and R_f independently are alkyl, unsaturated alkyl or aryl groups. Preferred substituents are OH, SH, OR_e, and SR_e where R_e is a lower alkyl, i.e., an alkyl group having from 1 to about 3 carbon atoms. Other preferred substituents are halogens, more preferably chlorine or bromine, and lower alkyl and unsaturated lower alkyl groups having from 1 to about 3 carbon atoms. Substituents include bridging groups between aromatic rings in the aryl group, such as $-CO_2-$, -CO-, -O-, -S-, -P-, -NH-, -CH=CH- and $-(CH_2)_\ell-$ where ℓ is an integer from 1 to about 5, and particularly $-CH_2-$. Examples of aryl groups having bridging substituents include

phenylbenzoate. Substituents also include moieties, such as $-(CH_2)_\ell-$, $-O-(CH_2)_\ell-$ or $-OCO-(CH_2)_\ell-$, where ℓ is an integer from about 2 to 7, as appropriate for the moiety, which bridge two ring atoms in a single aromatic ring as, for example, in a 1, 2, 3, 4-tetrahydronaphthalene group. Alkyl and unsaturated alkyl substituents of aryl groups can in turn optionally be substituted as described *supra* for substituted alkyl and unsaturated alkyl groups.

The terms "alkoxy group" and "thioalkoxy group" (also known as mercaptide groups, the sulfur analog of alkoxy groups) take their generally accepted meaning. Alkoxy groups include but are not limited to methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, sec-butoxy, isobutoxy, tert-butoxy, n-pentyloxy, neopentyloxy, 2-methylbutoxy, 1-methylbutoxy, 1-ethyl propoxy, 1,1 -dimethylpropoxy, n-hexyloxy, 1-methylpentyloxy, 2-methylpentyloxy, 3-methylpentyloxy, 4-methylpentyloxy, 3,3-dimethylbutoxy, 2,2-dimethoxybutoxy, 1-1-dimethylbutoxy, 2-ethylbutoxy, 1-ethylbutoxy, 1,3-dimethylbutoxy, n-pentyloxy, 5-methylhexyloxy, 4-methylhexyloxy, 3-methylhexyloxy, 2-methylhexyloxy, 1-methylhexyloxy, 3-ethylpentyloxy, 2-ethylpentyloxy, 1-ethylpentyloxy, 4,4-dimethylpentyloxy, 3,3-dimethylpentyloxy, 2,2-dimethylpentyloxy, 1,1-dimethylpentyloxy, n-octyloxy, 6-methylheptyloxy, 5-methylheptyloxy, 4-methylheptyloxy, 3-methylheptyloxy, 2-methylheptyloxy, 1-methylheptyloxy, 1-ethylhexyloxy, 1-propylpentyloxy, 3-ethylhexyloxy, 5,5-dimethylhexyloxy, 4,4-dimethylhexyloxy, 2,2-diethylbutoxy, 3,3-diethylbutoxy, 1-methyl-1-propylbutoxy, ethoxymethyl, n-propoxymethyl, isopropoxymethyl, sec-butoxymethyl, isobutoxymethyl, (1-ethyl propoxy)methyl, (2-ethylbutoxy)methyl, (1-ethylbutoxy)methyl, (2-ethylpentyloxy)methyl, (3-ethylpentyloxy)methyl, 2-methoxyethyl, 1-methoxyethyl, 2-ethoxyethyl, 3-methoxypropyl, 2-methoxypropyl, 1-methoxypropyl, 2-ethoxypropyl, 3-(n-propoxy)propyl, 4-methoxybutyl, 2-methoxybutyl, 4-ethoxybutyl, 2-ethoxybutyl, 5-ethoxypentyl, and 6-ethoxyhexyl. Thioalkoxy groups include but are not limited to the sulfur analogs of the alkoxy groups specifically listed *supra*.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted phenyl" means that the phenyl radical may or may not be

substituted and that the description includes both unsubstituted phenyl radicals and phenyl radicals wherein there is substitution.

"Amino acids" as used herein include naturally occurring and commercially available amino acids and optical isomers thereof. Typical natural and commercially available amino acids are glycine, alanine, serine, homoserine, threonine, valine, norvaline, leucine, isoleucine, norleucine, aspartic acid, glutamic acid, lysine, ornithine, histidine, arginine, cysteine, homocysteine, methionine, phenylalanine, homophenylalanine, phenylglycine, o-, m-, and p-tyrosine, tryptophan, glutamine, asparagine, proline and hydroxyproline. "Amino acid" as used herein includes amino acid residues and amino acid side chains. An "amino acid residue" is an amino acid radical --NHCH(R)C(O)--, wherein R is an amino acid side chain, except for the amino acid residues of proline and hydroxyproline which are --N(CH_2-CH_2-CH_2)CHC(O)-- and --N(CH-CHOHCH_2)CHC(O)–, respectively. An amino acid side chain is a radical found on the α-carbon of an α-amino acid as defined herein, where the radical is either hydrogen (side chain of glycine), methyl (side chain of alanine), or is a radical bonded to the α-carbon by a methylene (--CH_2--), or phenyl group.

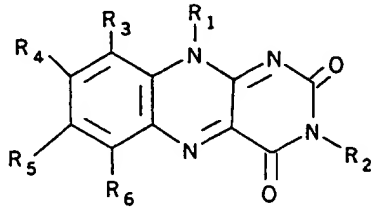
A protected glucose derivative takes its usual meaning in the art and includes a glucose molecule wherein some of the hydroxyl groups are substituted with acetate groups.

"Contacting" reaction components with each other refers to providing a medium and/or reaction chamber in which the reaction components are placed together so that they can react with each other. Preferably, the reaction components are suspended or dissolved in a carrier fluid which is a liquid medium. "Maintaining reaction components in contact" means keeping the components together in such a way that they can react with each other.

"Straight chain or cyclic saccharides" include mono- , di- and poly-, straight chain and cyclic saccharides that are optionally substituted with an amino group which is optionally acetylated. Straight chain saccharides that are useful in this invention include but are not limited to those molecules with a chain of 5 or 6 carbon atoms with one or more -OH groups attached, and either an aldehyde or ketone group. Cyclic saccharides are saccharides that are

in a ring form. Disaccharides are compounds wherein two monosaccharide groups are linked. Polysaccharides are compounds wherein more than two monosaccharide groups are linked. Specific examples of saccharides useful in this invention include glucose, ribose and glucosamine, among others.

"Isoalloxazine", "isoalloxazine derivative" or "core structure of isoalloxazine" include compounds that comprise the structure:



where R1-R6 are substituted with various substituents, as described elsewhere.

As used herein, the term "neutralization of a microorganism" or "neutralizing" means totally or partially preventing the microorganism from replicating, either by killing the microorganism or otherwise interfering with its ability to reproduce. A "neutralizer" is a compound that is capable of neutralizing a microorganism. The neutralizers useful in this invention include molecules with the core structure of isoalloxazine, as defined above. To "activate the microorganism neutralizer" is to expose the microorganism neutralizer to a triggering event that causes it to become active toward neutralizing microorganisms.

Microorganisms include viruses (both extracellular and intracellular), bacteria, bacteriophages, fungi, blood-transmitted parasites, and protozoa. Exemplary viruses include acquired immunodeficiency (HIV) virus, hepatitis A, B and C viruses, sinbis virus, cytomegalovirus, vesicular stomatitis virus, herpes simplex viruses, e.g. types I and II, human T-lymphotropic retroviruses, HTLV-III, lymphadenopathy virus LAV/IDAV, parvovirus, transfusion-transmitted (TT) virus, Epstein-Barr virus, and others known to the art. Bacteriophages include ΦX174, Φ6, λ, R17, T_4, and T_2. Exemplary bacteria include *P. aeruginosa*, *S. aureus*, *S. epidermis*, *L. monocytogenes*, *E. coli*, *K. pneumonia* and *S. marcescens*. Neutralization of white blood cells may be desirable when suppression of

immune or autoimmune response is desired, e.g., in processes involving transfusion of red cells, platelets or plasma when donor white blood cells may be present.

"Triggering event" refers to the stimulus that activates the microorganism neutralizer. Preferred triggering events include exposure of the neutralizer to an neutralization effective wavelength of light, or a pH sufficient to activate the neutralizer to neutralize microorganisms.

"Water soluble group" includes a group that, when included as a substituent on the neutralizer, imparts substantial solubility in water to the compound. Typically, the compound is soluble in water at a concentration of about 10 - 150 μM. Water soluble groups as referred to in this invention include, but are not limited to alcohols; polyalcohols; straight chain or cyclic saccharides; amines and polyamines; sulfate groups; phosphate groups; ascorbate groups; alkyl chains optionally substituted with -OH at any position; glycols, including polyethylene glycols, and polyethers.

The term "biologically active" means capable of effecting a change in a living organism or component thereof. "Biologically active" with respect to "biologically active protein" as referred to herein does not refer to proteins which are part of the microorganisms being neutralized. Similarly, "non-toxic" with respect to the neutralizers means low or no toxicity to humans and other mammals, and does not mean non-toxic to the microorganisms being neutralized. "Substantial destruction" of biological activity means at least as much destruction as is caused by porphyrin and porphyrin derivatives, metabolites and precursors which are known to have a damaging effect on biologically active proteins and cells of humans and mammals. Similarly, "substantially non-toxic" means less toxic than porphyrin, porphyrin derivatives, metabolites and precursors that are known for blood sterilization. Preferably, neutralizers are less toxic than porphyrin, porphyrin derivatives, metabolites and precursors that are known for blood sterilization.

The term "blood product" as used herein includes blood constituents and therapeutic protein compositions containing proteins derived from blood as defined above. Fluids containing biologically active proteins other than those derived from blood may also be

treated by the methods of this invention. Such fluids may also contain one or more components selected from the group consisting of protein, e.g. biologically active protein such as a therapeutic protein, blood and blood constituents, without destroying the biological activity of such components.

Decontamination methods of this invention using isoalloxazine derivatives as defined above do not substantially destroy the biological activity of fluid components other than microorganisms. As much biological activity of these components as possible is retained, although in certain instances, when the methods are optimized, some loss of biological activity, e.g., denaturization of protein components, must be balanced against effective decontamination of the fluid. So long as fluid components retain sufficient biological activity to be useful for their intended or natural purposes, their biological activities are not considered to be substantially destroyed.

"Decomposition" of the neutralizer upon exposure to light refers to the chemical transformation of the neutralizer into new compounds. An example of decomposition of the neutralizer is the production of lumichrome upon exposure of riboflavin to visible light.

A "photosensitizer" is defined as any compound which absorbs radiation of one or more defined wavelengths and subsequently utilizes the absorbed energy to carry out a chemical process. Photosensitizers of this invention may include compounds which preferentially adsorb to nucleic acids, thus focusing their photodynamic effect upon microorganisms and viruses with little or no effect upon accompanying cells or proteins. Other photosensitizers of this invention are also useful, such as those using singlet oxygen-dependent mechanisms.

An "alkylating agent" is a compound that reacts with amino acid residues and nucleic bases and inhibits replication of microorganisms.

DETAILED DESCRIPTION OF THE INVENTION

The contaminant neutralizers of the invention neutralize microorganisms by exposure to a triggering event, preferrably by exposure to an activation-effective wavelength of light in

the uv/visible region of the spectrum or an activation-effective pH. The neutralizer must be one which does not substantially destroy desired components of the fluid being decontaminated, and also preferably which does not degrade into products which substantially destroy desired components or have significant toxicity or substantially decompose into ultraviolet light absorbing compounds.

In embodiments of the invention using light as a triggering event, the fluid containing an appropriate concentration of the neutralizer is exposed to photoradiation of the appropriate wavelength to activate the neutralizer, using an amount of photoradiation sufficient to activate the neutralizer, but less than that which would cause substantial damage to the biological components or substantially interfere with biological activity of other proteins present in the fluid. The wavelength of light used and the amount of radiation used will depend on the neutralizer selected, as is known to the art or readily determinable without undue experimentation by one of ordinary skill in the art, using literature sources or direct measurement. Preferably the light source is a uv/visible light source providing 320 nm to about 700 nm, and more preferably about 365 nm to about 650 nm of radiation. The amount of neutralizer to be mixed with the fluid will be an amount sufficient to adequately neutralize microorganisms therein. Preferably the neutralizer is soluble in the fluid and present in an amount less than the upper solubility limit of the neutralizer in the fluid. As taught herein, optimal concentrations for desired neutralizers may be readily determined by those skilled in the art without undue experimentation. Preferably, the smallest effecacious concentration of neutralizer is used. Typically, the neutralizer is used in a concentration of at least about 1 μM up to the solubility of the neutralizer in the fluid, and typically the concentration of neutralizer is about 10 μM. Other concentrations are also able to be used. An excess of neutralizer may be present in the solution. The neutralizer may also be used in a suspension, where the neutralizer is not soluble in the fluid, provided that adequate mixing is provided to contact the neutralizer with the fluid. The neutralizer may also be removed from the fluid prior to administration of the fluid to a patient. All other parameters that may be involved in a decontamination system, including appropriate temperatures for the reaction of the neutralizer as well as the ranges of temperature, photoradiation intensity and duration, and neutralizer concentration which will optimize microbial neutralization and minimize damage to desired proteins and/or cellular components in the fluid are also easily determined as is

known in the art or readily determinable without undue experimentation by one of ordinary skill in the art, using literature sources or direct measurement.

In embodiments of this invention using pH to neutralize the contaminants, the appropriate pH, concentration of neutralizer that is effective, and other parameters are determined by means known to one of ordinary skill in the art. In particular embodiments, contacting the contaminant neutralizer with the fluid containing microorganisms to be neutralized may be sufficient to activate the contaminant neutralizer (i.e., the triggering event when pH is used to activate the microorganism neutralizer may not need to be externally applied). An effective concentration is generally from about 10 - 100 μM. A pH of about 5 to about 8 is generally effective to activate the neutralizer. Other concentrations and pH's may be used.

A solution or suspension of contaminant neutralizer may be prepared and stored and when desired, used by contacting with fluid or other substance containing contaminants and exposing to a triggering event.

Once such system requirements have been determined, the appropriate apparatus may be designed. Batch or flow-through systems may be used, for example. The isoalloxazine derivatives of this invention can be used in the decontamination systems described in U.S. Patent Nos. 5,290,221, 5,536,238, 5,290,221 and 5,536,238, and U.S. Patent Application Nos. 09/119,666 and 09/357,188. In general, the fluid to be decontaminated is mixed with neutralizer. If light is used to neutralize the contaminants, the fluid and neutralizer are irradiated with a sufficient amount of photoradiation at an appropriate wavelength to activate the neutralizer to react with microorganisms in the fluid such that microorganisms in the fluid are neutralized. If pH is used to neutralize the contaminants, the pH of the fluid and neutralizer is changed, if necessary, by any means known in the art.

Examples of materials which may be treated by the methods of this invention are whole blood and aqueous compositions containing biologically active proteins derived from blood or blood constituents. Packed red cells, platelets and plasma (fresh or fresh frozen plasma) are exemplary of such blood constituents. In addition, therapeutic protein

compositions containing proteins derived from blood, such as fluids containing biologically active protein useful in the treatment of medical disorders, e.g., factor VIII, Von Willebrand factor, factor IX, factor X, factor XI, Hageman factor, prothrombin, anti-thrombin III, fibronectin, plasminogen, plasma protein fraction, immune serum globulin, modified immune globulin, albumin, plasma growth hormone, somatomedin, plasminogen streptokinase complex, ceruloplasmin, transferrin, haptoglobin, antitrypsin and prekallikrein may be treated by the decontamination methods of this invention. Other fluids which could benefit from the treatment of this invention are peritoneal solutions used for peritoneal dialysis which are sometimes contaminated during connection, leading to peritoneal infections.

This method is also useful for treating other fluids including fluids which are meant for nourishment of humans or animals such as water, fruit, juices, milk, broths, soups and the like. The method is also useful for treating parenteral solutions. This invention may also be used to treat surfaces, as described in United States Patent Application No. 09/119,666. The isoalloxazine derivative compounds of this invention may also coat surfaces such as blood or peritoneal dialysis tubing sets to assure sterile connections and sterile docking.

The neutralizer may be applied in a suitable carrier such as water or a solution containing other treatment additives, by spraying, dipping, wiping on, or by other means known to the art. The amount of neutralizer and the conditions to activate the neutralizer required for treatment will be readily determined by one of skill in the art without undue experimentation depending on the level of contamination and the material being treated.

The activated neutralizer is capable of neutralizing the microorganisms present, such as by interfering to prevent their replication. This may occur with activation of the molecule with uv/visible light, or may occur by the nature of the substituent on the isoalloxazine core and an alteration of the pH of the system in the absence of light. Specificity of action of the neutralizer may be conferred by the close proximity of the neutralizer to the nucleic acid of the microorganism and this may result from binding of the neutralizer to the nucleic acid. "Nucleic acid" includes ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Other Neutralizers may act by binding to cell membranes or by other mechanisms. The neutralizer

may also be targeted to the microorganism to be neutralized by covalently coupling to an antibody, preferably a specific monoclonal antibody to the microorganism.

Enhancers may also be added to the fluid to make the process more efficient and selective. Such enhancers include antioxidants or other agents to prevent damage to desired fluid components or to improve the rate of neutralization of microorganisms and are exemplified by adenine, histidine, cysteine, tyrosine, tryptophan, ascorbate, N-acetyl-L-cysteine, propyl gallate, glutathione, mercaptopropionylglycine, dithiothreotol, nicotinamide, BHT, BHA, lysine, serine, methionine, glucose, mannitol, trolox, glycerol, and mixtures thereof.

The use of the compounds of this invention to neutralize microorganisms requires mixing or contacting the isoalloxazine derivative with the material to be decontaminated. Mixing or contacting may be done by simply adding the neutralizer or a solution containing the neutralizer to a fluid to be decontaminated. In one embodiment using light to neutralize the microorganisms, the material to be decontaminated to which a light-triggered neutralizer has been added is flowed past a photoradiation source, and the flow of the material generally provides sufficient turbulence to distribute the neutralizer throughout the fluid to be decontaminated. In another embodiment, the fluid and light-triggered neutralizer are placed in a photopermeable container and irradiated in batch mode, preferably while agitating the container to fully distribute the photosensitizer and expose all the fluid to the radiation. In another embodiment, insoluble materials may be used in the process of this invention, for example, by suspending the isoalloxazine derivative in the biological fluid and exposing the fluid and isoalloxazine derivative to the triggering event. In another embodiment, the pH-triggered compound is placed in contact with the fluid to be treated. In some embodiments using a pH-triggered compound, the pH of the fluid-compound mixture will require changing in order to trigger neutralization by means known to one of ordinary skill in the art, such as the use of acid or base.

EXAMPLES

Example 1. Absorbance Profile of isoalloxazine derivative

A sample of an isoalloxazine derivative is analyzed using a scanning UV spectrophotometer over the region 200 to 900 nm. For analysis, the sample is dissolved in distilled water. An absorption spectrum is obtained, and extinction coefficients at the absorbance maxima and other wavelengths of interest are determined. From the absorption spectrum and extinction coefficients, appropriate wavelengths for irradiation are determined. An appropriate wavelength is one at which the extinction coefficient is sufficient to ensure adequate activation of the sensitizer in solution.

Example 2. Neutralization of microorganisms with isoalloxazine derivatives using light

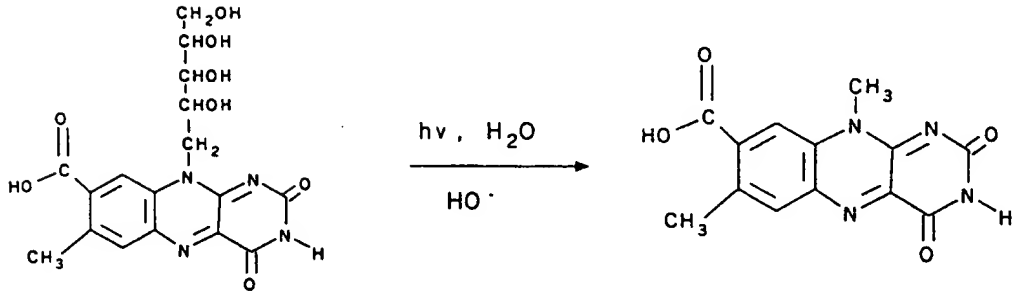
7, 8, 10-trimethyl, 3-sulfonyl isoalloxazine is dissolved in blood at a concentration of 10 μM. The sample is spiked with a representative microorganism. Flow of the sample through an irradiation chamber is maintained and the sample is irradiated with a neutralization-effective level of light at a wavelength determined to be appropriate for neutralization, as described above. The extent of neutralization of the microorganism is measured by methods known in the art.

Example 3. pH sensitivity studies

7-chloroethylamino-8,10-methyl isoalloxazine is dissolved in blood at concentrations of 10 - 100 μM. The solutions are spiked with a representative microorganism. Aliquots are removed and the pH of different aliquots is adjusted to 1.0, 3.0, 5.0, 7.0, 9.0 with sodium carbonates. The solutions are mixed to distribute the components. The neutralization results are determined as described above.

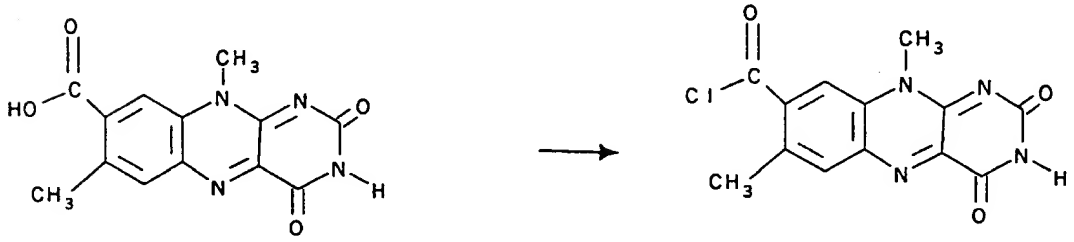
Synthesis

Carboxyriboflavin (1, McCormick, D. (1970) J. Heter. Chem. 7:447) is photolyzed in aqueous alkali to form a carboxylumiflavine (2).

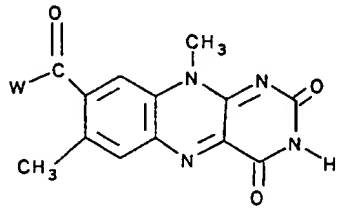


1 2

Compound 2 is converted to an acid chloride 3 with oxallylchloride.



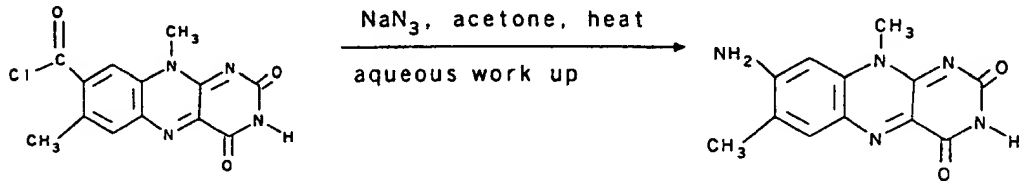
2 3



4

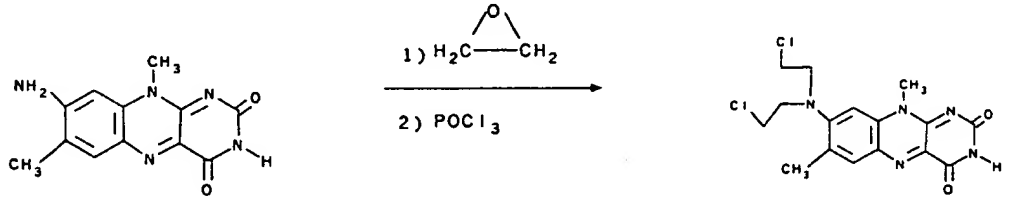
Compound 3 is reacted with ascorbate ion, glucosamine, a protected glucose derivative or di or triethylene glycol to form a water soluble derivative 4 where the light sensitive water soluble moiety W is far removed from the amide containing ring.

Compound 3 is reacted with sodium azide in acetone to effect a Curtius Rearrangement. This forms compound 5, upon work-up. This reaction effectively replaces a CO_2H group with an NH_2 group.



3 **5**

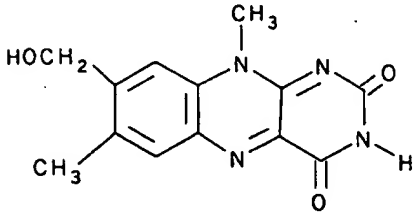
Lumiflavine amine 5 is converted into compound 6 by the procedure of J.L. Everett, et al. (1953) J. Chem. Soc., p 2386.



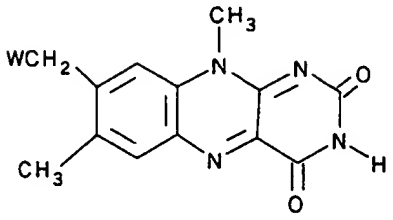
5 **6**

One of the chlorines from 6 will be replaced with W to impart water solubility to the compound.

Riboflavin methanol is synthesized by the method of McCormick and upon photolysis it will yield lumiflavine methanol 7.



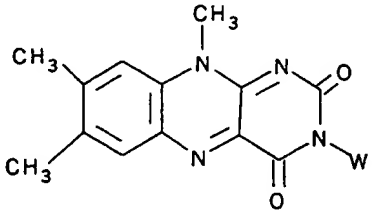
7



8

The hydroxyl group is replaced with a water soluble group (e.g., W, 8) as described earlier.

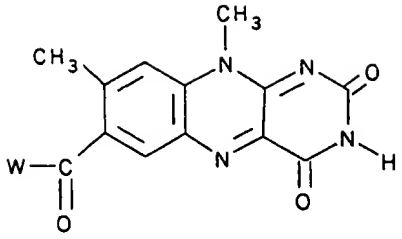
The N-3 (R2) of lumiflavine is alkylated using the method of P. Hemmerich (1964) Helv. Chim. Acta 47:464. This method is adapted to place water soluble groups at (R2) (e.g., 9).



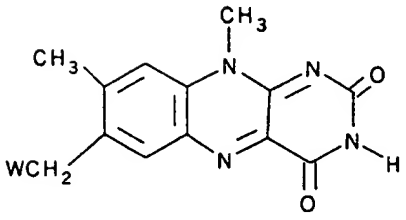
9

This lumiflavine will be water soluble, absorb visible light, and should not break down upon photolysis with visible light.

The corresponding series 10 and 11 are formed by application of known reactions.



10



11

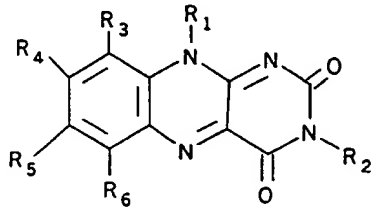
All compounds of this invention may be prepared by the methods above or by methods well known in the art, or by adapting the methods above or methods well known in the art. In addition, reactants specified herein may be substituted for others that produce a similar function.

Although the description above contains many specificities, these should not be construed as limiting the scope of the invention but as merely providing illustrations of some of the presently-preferred embodiments of this invention. Thus, the scope of the invention should be determined by the appended claims and their legal equivalents, rather than by the examples given.

CLAIMS

1. A method for treating a fluid to neutralize microorganisms which may be present therein, said fluid containing one or more components selected from the group consisting of protein, blood, and blood constituents, said method comprising:

(a) adding to said fluid a neutralization-effective amount of a microorganism neutralizer of formula:



wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing, and $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20;

provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O and R1, R4, R5 are not all methyl groups when R2, R3 and R6 are hydrogen;

(b) exposing the fluid of step (a) to a triggering event whereby said microorganisms are neutralized.

2. The method of claim 1, wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted alcohol, straight chain or cyclic saccharide, amino acid, amine, polyamine, polyether, polyalcohol, sulfate, phosphate, carbonyl, glycol, halogen selected from the group

consisting of chlorine, bromine and iodine, aldehyde, ketone, carboxylic acid and ascorbate.

3. The method of claim 1, wherein said triggering event is photoradiation sufficient to activate the microorganism neutralizer.

4. The method of claim 1, wherein said triggering event is a pH sufficient to activate the microorganism neutralizer.

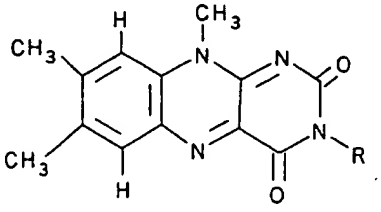
5. The method of claim 4, wherein said pH is between about 5 and about 8.

6. The method of claim 1 wherein said microorganisms are selected from the group consisting of bacteria, bacteriophages, and intracellular and extracellular viruses.

7. The method of claim 1 wherein said microorganisms are bacteria.

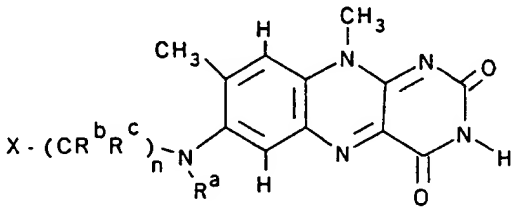
8. The method of claim 1, wherein said microorganisms are selected from the group consisting of HIV viruses, hepatitis viruses, sindbis virus, cytomegalovirus, vesicular stomatitis virus, herpes simplex viruses, vaccinia virus, human T-lymphotropic retroviruses, HTLV-III, lymphadenopahy virus LAV/IDAV, parvovirus, transfusion-transmitted (TT) virus, Epstein-Barr virus, bacteriophages ΦX174, Φ6, λ, R17, T_4, T_2, *P. aeruginosa, S. aureus, S. epidermidis, L. monocytogenes, E. coli, K. pneumoniae* and *S. marcescens*.

9. The method of claim 1, wherein said microorganism neutralizer is



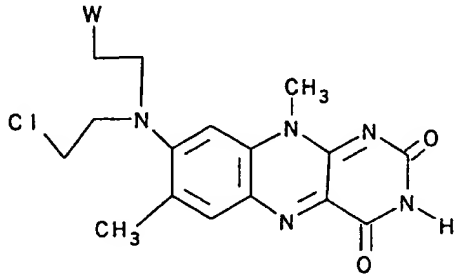
wherein R is selected from the group consisting of ascorbate, alcohol, polyalcohol, amine, polyamine, straight chain or cyclic saccharides, sulfates, phosphates, polyethylene glycols, and polyethers.

10. The method of claim 1, wherein said microorganism neutralizer is



wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20.

11. The method of claim 1 , wherein said microorganism neutralizer is



wherein W is a water soluble group.

12. The method of claim 1, wherein said fluid comprises blood constituents.

13. The method of claim 1, wherein said fluid comprises whole blood.

14. The method of claim 1, wherein said fluid comprises a separated blood product.

15. The method of claim 1, wherein said fluid comprises platelets separated from whole blood.

16. The method of claim 1, wherein said fluid comprises red blood cells separated from whole blood.

17. The method of claim 1, wherein said fluid comprises serum separated from whole blood.

18. The method of claim 1, wherein said fluid comprises plasma separated from whole blood.

19. The method of claim 1, wherein said fluid comprises a therapeutic protein composition.

20. The method of claim 1, wherein said fluid contains a biologically-active protein selected from the group consisting of: factor VIII, Von Willebrand factor, factor IX, factor X, factor XI, Hageman factor, prothrombin, anti-thrombin III, fibronectin, plasminogen, plasma protein fraction, peritoneal dialysis solutions, immune serum globulin, modified immune globulin, albumin, plasma growth hormone, somatomedin, plasminogen streptokinase complex, ceruloplasmin, transferrin, haptoglobin, antitrypsin and prekallikrein.

21. The method of claim 1, wherein said microorganism neutralizer is added to anticoagulant and said anticoagulant is added to said fluid.

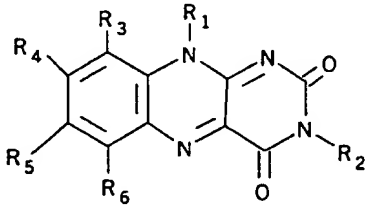
22. The method of claim 1, wherein an enhancer is added to said fluid prior to exposing said fluid to said triggering event.

23. The method of claim 22, wherein said enhancer is selected from the group consisting of adenine, histidine, cysteine, tyrosine, tryptophan, ascorbate, N-acetyl-L-cysteine, propyl gallate, glutathione, mercaptopropionylglycine, dithiothreotol, nicotinamide, BHT, BHA, lysine, serine, methionine, glucose, mannitol, trolox, glycerol, and mixtures thereof.

24. The method of claim 1 , wherein if said microorganism neutralizer produces photolytic products, the photolytic products are of low or no toxicity to humans or animals.

25. A method for treating a fluid to neutralize microorganisms which may be present therein, said method comprising:

(a) adding to said fluid an neutralization-effective amount of a microorganism neutralizer of formula:



wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing;

and $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20; provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O and R1, R4, R5 are not all methyl groups when R2, R3 and R6 are all hydrogen;

(b) exposing the fluid of step (a) to a triggering event whereby said microorganisms are neutralized.

26. The method of claim 25, wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen; optionally substituted alcohol, polyalcohol, straight chain or cyclic saccharide, amino acid, ether, polyether, amine, polyamine, sulfate, phosphate, carbonyl, glycol, halogen selected from the group chlorine, bromine and iodine, aldehyde, ketone, carboxylic acid and ascorbate.

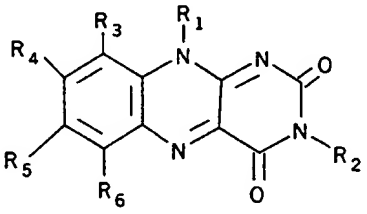
27. The method of claim 25, wherein said fluid is a food product.

28. The method of claim 25, wherein said fluid is a drink meant for human or animal consumption.

29. The method of claim 25, wherein said fluid is a peritoneal dialysis solution.

30. A method of neutralizing microorganisms on a surface, comprising:

(a) applying to said surface an neutralization-effective amount of a compound of formula:



wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing;

and -NR^a-$(CR^bR^c)_n$-X wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20;

provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O and R1, R4, R5 are not all methyl groups when R2, R3 and R6 are all hydrogen;

(b) exposing said surface to a triggering event whereby said microorganisms are neutralized

31. The method of claim 30, wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen; optionally substituted alcohol, polyalcohol, straight chain or cyclic saccharide, amino acid, ether, polyether, amine, polyamine, sulfate, phosphate, carbonyl, glycol, halogen selected from the group chlorine, bromine and iodine, aldehyde, ketone, carboxylic acid and ascorbate.

32. The method of claim 30, wherein said surface is a food surface.

33. The method of claim 30, wherein said surface is the surface of an animal carcass.

34. The method of claim 30, wherein said surface is a food-preparation surface.

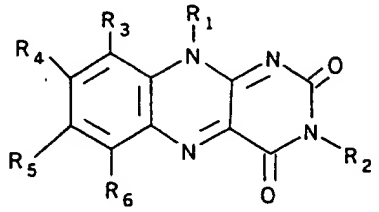
35. The method of claim 30, wherein said surface is a surface of a bathing or washing vessel.

36. The method of claim 30, wherein said surface is a wound surface.

37. A fluid comprising biologically active protein, blood or blood constituents, and microorganism neutralizer, made by the method of claim 1.

38. A blood product comprising a microorganism neutralizer, made by the method of claim 1.

39. A compound having the structure:

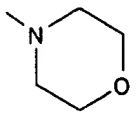


wherein R1, R2, R3, R4, R5 and R6 are, independently from one another, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing;

and $-NR^a-(CR^bR^c)_n-X$ wherein X is a halogen selected from the group consisting of chlorine, bromine and iodine, R^a, R^b and R^c are, independently of each other, selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, and halogen selected from the group consisting of chlorine, bromine and iodine, and n is an integer from 0 to 20;

provided that R1 is not -OH or a straight chain alkyl group where the second carbon of the chain is substituted with -OH or =O; and R1 is not a 2-, 3-, 4- or 5- carbon straight chain alkyl that terminates in -OH, -COH, or -H when R2, R3 and R6 are H, and R4 and R5 are CH_3; R1 is not - CH_2CH_2-$(CHOH)_2$-CH_3 or -CH_2CH_2-$(CHOH)_2$ -CH_2SO_4 or 1'-D-sorbityl or 1'-D-dulcityl or 1'-D-rhamnityl or 1'-D,L-glyceryl or -CH_2-O-C(O)-CH_3 or -CH_2-O-C(O)-CH_2CH_3 or 2', 3', 4', 5'-di-O-isopropyridene-riboflavin or 8-aminooctyl when R2, R3 and R6 are H and R4 and R5 are CH_3; R1 is not 1'-D-sorbityl or 1'-D-dulcityl when R4 and R5 are both chlorines and when R2, R3 and R6 are all hydrogens; R5 is not ethyl or chloro when R1 and R4 are methyl and R2, R3 and R6 are all hydrogens; R4 and R5 are not both methoxy or both tetramethylene when R1 is methyl and R2, R3 and R6 are all hydrogens; R2 is not

$-CH_2CH_2NH$ when R1, R4 and R5 are CH_3 and R3 and R6 are H; R2 is not



when R1, R4 and R5 are CH_3 and R3 and R6 are H; R5 is not chloro when R4 is methoxy and R1 is ethyl-2'N-pyrrolidino and R2, R3, and R6 are hydrogen; R1 is not N,N-dimethylaminopropyl or N,N-diethylaminoethyl when R5 is chloro or methyl and R2, R3, R4 and R6 are hydrogen; R3 is not $-NH(CH_2CH_2)Cl$ when R6 is -NH2 and R1, R2, R4 and R5 are H; R1, R4, R5 are not all methyl groups when all of R2, R3 and R6 are hydrogens; R1, R4, R5 and R2 are not all methyl groups when R3 and R6 are hydrogens; R2 is not carboxymethyl when R1, R4 and R5 are methyl and R3 and R6 are hydrogen; R4 is not -NH2 when R1 and R5 are methyl and R2, R3 and R6 are all hydrogen; R1 is not a phenyl group when R4 and R5 are methyl and R2, R3 and R6 are all H; R1 is not methyl or N,N-dimethylaminoethyl when all of R2, R3, R4, R5 and R6 are hydrogen; R2, R4, R5 are not all methyl when R1 is acetoxyethyl and R3 and R6 are hydrogen; R5 is not methyl when R1 is N,N-diethylaminoethyl and R2, R3, R4 and R6 are all hydrogen; R4 and R5 are not both chlorine when R1 is methyl and R2, R3 and R6 are all hydrogen; R1 is not ethyl, β-chloroethyl, n-butyl, anilino, benzyl, phenyl, p-tolyl or p-anisyl when R5 is NH_2 and R2, R3, R4 and R6 are all hydrogen; and R4 is not chlorine when R1 is N,N-dimethylaminopropyl and R2, R3, R5 and R6 are all hydrogen.

40. The compound of claim 39, wherein a plurality of R1, R2, R3, R4, R5 and R6 are neither CH_3 nor H.

41. The compound of claim 40, wherein a plurality of R2, R3, R4, R5 and R6 are neither H nor CH_3.

42. The compound of claim 40, wherein a R1, R2, R3, R4, R5 and R6 that is neither CH_3 nor H imparts substantial water solubility to the compound.

43. The compound of claim 42, wherein said R1, R2, R3, R4, R5 and R6 is selected from the group consisting of:
alcohols; polyalcohols; straight chain or cyclic saccharides; ether; polyether; amines; polyamines; sulfate groups; phosphate groups; ascorbate groups; alkyl chains optionally substituted with -OH at any position; glycols; and polyethers.

44. The compound of claim 43, wherein R1 is not CH_2-$(CH_2OH)_3$-CH_2OH.

45. The compound of claim 43, wherein R1 is -CH_2-$(CH_2OH)_3$-CH_2OH.

46. The compound of claim 43, wherein R3 and R6 are H.

47. The compound of claim 40, wherein at least one of R1, R2, R3, R4, R5 and R6 contains a halogen selected from the group consisting of chlorine, bromine and iodine.

48. The compound of claim 47, wherein at least one of R1, R2, R3, R4, R5 and R6 is -(CH_2)n-X, wherein n is either 1 or 2, and X is a halogen selected from the group consisting of chlorine, bromine and iodine.

49. The compound of claim 47, wherein at least one of the halogenated R1, R2, R3, R4, R5 and R6 is -NR(CH_2)n-X, wherein R is hydrogen or straight chain alkyl group consisting of one to 6 carbon atoms, n is an integer from 0 to 6, and X is selected from the group consisting of chlorine, bromine and iodine.

50. The compound of claim 49 wherein R4 or R5 is -NR(CH_2)n-X, wherein R is hydrogen or straight chain alkyl group consisting of one to 6 carbon atoms, n is an integer from 0 to 6, and X is selected from the group consisting of chlorine, bromine and iodine.

51. The compound of claim 39, wherein one of R1, R2, R3, R4, R5 and R6 is neither CH_3 nor H.

52. The compound of claim 51, wherein the R1, R2, R3, R4, R5 and R6 that is neither CH_3 nor H imparts substantial water solubility to the compound.

53. The compound of claim 52, wherein the R1, R2, R3, R4, R5 and R6 is selected from the group consisting of:

alcohols; polyalcohols; straight chain or cyclic saccharides; amines and polyamines; sulfate groups; phosphate groups; ascorbate groups; alkyl chains optionally substituted with -OH at any position; glycols; ethers and polyethers.

54. The compound of claim 53, wherein R1 is not CH_2-$(CH_2OH)_3$-CH_2OH.

55. The compound of claim 53, wherein the R1, R2, R3, R4, R5 and R6 that is neither H nor CH_3 is R2, R3, R4, R5 or R6.

56. The compound of claim 53, wherein R3 and R6 are H.

57. The compound of claim 51, wherein one of R1, R2, R3, R4, R5 and R6 is halogenated, wherein the halogen is selected from the group consisting of chlorine, bromine and iodine.

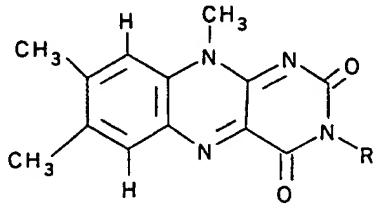
58. The compound of claim 57, wherein one of R1, R2, R3, R4, R5 and R6 is -(CH_2)n-X, wherein n is either 1 or 2, X is a halogen selected from the group consisting of chlorine, bromine and iodine.

59. The compound of claim 57, wherein one of R1, R2, R3, R4, R5 and R6 is -NR(CH_2)n-X, wherein R is hydrogen or straight chain alkyl group consisting of one to 6 carbon atoms, n is an integer from 0 to 6, and X is selected from the group consisting of chlorine, bromine and iodine.

60. The compound of claim 59 wherein R4 or R5 is -NR(CH_2)n-X, wherein R is hydrogen or straight chain alkyl group consisting of one to 6 carbon atoms, n is an integer from 0 to 6, and X is selected from the group consisting of chlorine, bromine and iodine.

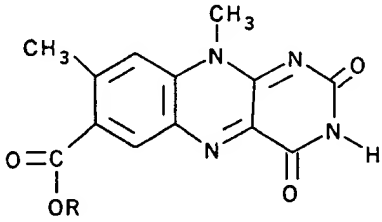
61. The compound of claim 39 wherein at least one of R1, R2, R3, R4, R5 and R6 are branched or unbranched C1 to C20 alkyl groups substituted with at least one -OH group.

62. The compound of claim 39 having the structure:



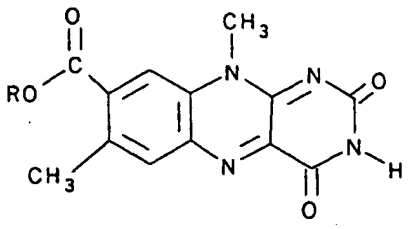
wherein R is selected from the group consisting of ascorbate, alcohol, polyalcohol, amine or polyamines, straight chain or cyclic saccharides, sulfates, phosphates, polyethylene glycols and polyethers.

63. The compound of claim 39 having the structure



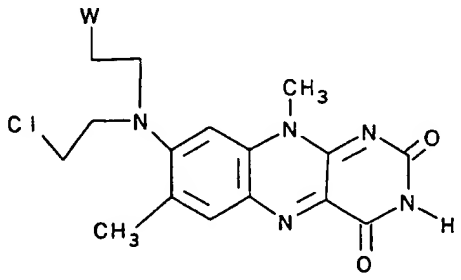
wherein R is selected from the group consisting of hydrogen and optionally substituted straight chain or branched alkyl having from 1 to 20 carbon atoms.

64. The compound of claim 39 having the structure



wherein R is selected from the group consisting of hydrogen and optionally substituted straight chain or branched alkyl having from 1 to 20 carbon atoms.

65. The compound of claim 39, having the structure:

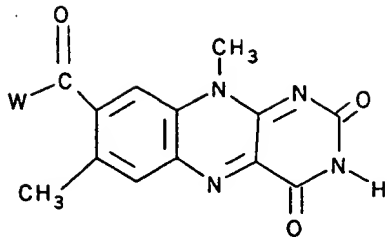


wherein W is a water soluble group.

66. The compound of claim 39 wherein at least one of R1, R2, R3, R4, R5 and R6 are alkylating agents.

67. The compound of claim 39 wherein at least one of R1, R2, R3, R4, R5 and R6 are substituents that cause the compound to be substantially nonreactive to microorganisms at substantially neutral pH and active toward microorganism neutralization at the pH of the biological fluid.

68. A method of making a compound having structure:



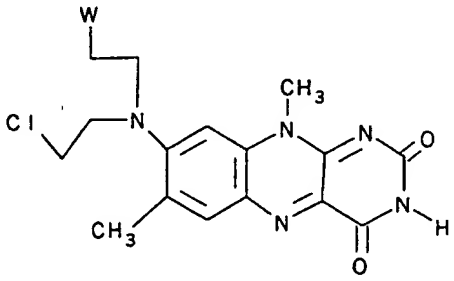
wherein W is a water soluble group, comprising:

(a) photolyzing carboxyriboflavin;

(b) reacting (a) with oxallylchloride;

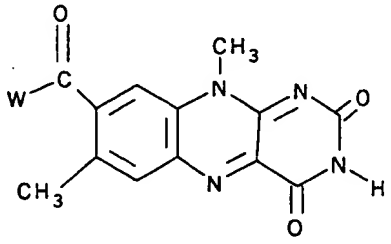
(c) reacting (b) with a member of the group consisting of ascorbate, glucosamine, protected glucose derivatives, diethylene glycol and triethylene glycol.

69. A method of making a compound having the structure:

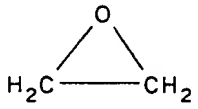


where W is a water soluble group,
comprising:

(a) contacting



with sodium azide;

(b) reacting (a) with  and $POCl_3$.

(c) reacting (b) with a water solubilizing group.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 00/25213

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L2/08 A61L2/10 C07D475/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 196 515 A (BAXTER TRAVENOL LAB) 8 October 1986 (1986-10-08) cited in the application claims	1-38
A	EP 0 679 398 A (EISAI CO LTD) 2 November 1995 (1995-11-02) claims	1,39
A	WO 96 39816 A (CREDIT MANAGERS ASS OF CALIFOR) 19 December 1996 (1996-12-19) claims	1-38
P,A	WO 00 04930 A (COBE LAB) 3 February 2000 (2000-02-03) cited in the application claims	1-38

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2001	19/01/2001

Name and mailing address of the ISA	Authorized officer
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		NO 20001440 A	19-05-2000